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Examining the mechanistic roles of Integrin Alpha-6 in cancer metastasis.

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Examining the mechanistic roles of Integrin Alpha-6 in cancer metastasis.

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in *Biology*.

By
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Under the mentorship of *Dr. Vinoth Sittaramane.*

ABSTRACT

Metastasis- the spread of cancer cells from the primary tumor to the surrounding tissues- is responsible for 90% of cancer deaths. Integrin alpha-6 (ITGA6) is a specific transmembrane cell surface protein that functions in cell surface adhesion and signaling. ITGA6 is upregulated in many types of cancers and promotes the migration and invasion of cancer cells to surrounding tissues. It is my objective to determine the mechanism through which ITGA6 facilitates the migration of cancer cells through the extracellular matrix (ECM). These experiments helped to establish the role of ITGA6 in tumor development and provide focus for possible chemotherapeutic treatment. Using an in-vitro cell migration assay I observed the varying rates of invasion through the ECM by PC3 cancer cells transfected to express various amounts of ITGA6. High-resolution imaging techniques were used in coordination with cell counting software to examine the number of PC3 cells that had migrated across the laminin-coated membrane. I found that PC3 cells with ITGA6 knockdown had lower rates of cell migration compared to untreated PC3 cells while PC3 cells with ITGA6 overexpression had the greatest rate of invasion through the ECM. Based on my findings, future studies can aim to identify possible alternative pathways of ITGA6 function and to develop targeted cancer therapeutic strategies.

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INTRODUCTION

Cancer

Cancer is a highly complex and variable disease caused by a malfunctioning of our own cells. There are many different types of cancer, but they all share a common feature of unregulated proliferation. Normally, the division of cells is a highly regulated and controlled cycle; however, cancer cells develop a mutation in their cell cycle allowing them to replicate unimpeded. Cancer arises from a mutated gene known as an oncogene which in certain circumstances can transform a normal cell into a tumor cell. Abnormal cells that do not function properly normally undergo apoptosis and are replaced, but certain mutations can allow these cells to bypass this mechanism.

Cancers develop and evolve through clonal expansion and clonal selection of abnormal cells. The accumulation of mutations gives cancers unique traits including resistance to anti-growth signals, evading apoptosis, inducing angiogenesis, tissue metastasis, and more (Hanahan & Weinberg, 2011). Of these, cancer metastasis is of leading concern. According to statistics published by the National Cancer Institute in 2016, approximately 455 people per 100,000 are at risk of developing cancer, and of these 455 people, 171 will die because of it. Of these cancer deaths, it is estimated that approximately 90% are caused by metastasis. Creating efficient cancer diagnostic techniques as well as developing targeted cancer therapeutic treatments is of greatest concern within the field of cancer research. Cancer metastasis is increasingly fatal because as secondary tumors initiate and grow in different tissues/organs they interrupt the body's normal physiologic function which can lead to organ failure. Cancer researchers are focused on investigating the biological pathways of cells and the mechanisms of tumorigenesis. Understanding the mechanisms that initiate

Figure 1. The hallmarks of cancer; the acquired mutations of abnormal cells that contribute to the development of a tumor (Hannan & Weinberg, 2011).

and facilitate cancer cell metastasis is of high priority in order to identify potential drug targets to prevent cancer metastasis, giving cancer patients better prognoses.

Integrins

Studies by Itou et al. in 2017 suggest that integrins play an important role in cell migration. Integrins are transmembrane proteins that function mechanically by attaching the cell cytoskeleton to the extracellular matrix (ECM). The integrin molecule is composed of two transmembrane glycoprotein subunits; alpha(α) and beta(β). The formation of a heterodimer between the alpha and beta subunits forms an integrin that interacts with extracellular matrix proteins, including laminin family proteins. There are 24 different types of integrin proteins in mammals, determined by the different combinations of alpha and beta subunits. With specific functions, these integrins create a bridge between the ECM and cytoskeleton of cells to detect changes in the environment, enabling cells to react to external signals. Integrins are involved in multiple developmental processes including cell differentiation, cell adhesion, cell migration, and cell proliferation (Bianconi, Unseld, Prager, 2016). In cancer, malignant cells manipulate these functional mechanisms.

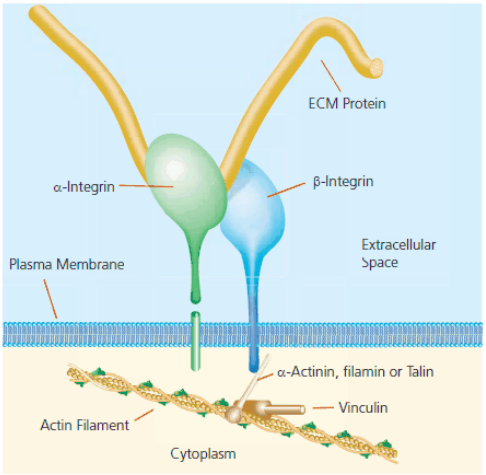

Figure 2. Integrin Structure: composed of one of 18 alpha-subunits and one of 8 beta-subunits.

Unlike mesenchymal cells, epithelial cells have the ability to migrate only during development and tissue repair. For a cancer cell to gain migratory ability, the epithelial cell must undergo epithelial to mesenchymal transition (EMT). Researchers are investigating possible mechanisms by which integrin expression facilitates EMT, initiating tumor invasion. The integrin subunit alpha-6 (ITGA6) has been observed to promote tumorigenesis; ITGA6 is a protein coding gene that encodes a family of proteins on the alpha-6 subunit. ITGA6 is upregulated in many types of cancers (**Figure 3**) and has been shown to promote the migration and invasion of cancer cells to surrounding tissues, but the functional mechanism remains unclear. In 2019, researchers used mass

spectrometry to find that ITGA6 was more highly expressed in a highly invasive pancreatic cancer cell line than compared to a less invasive cell line. They concluded that a higher expression of ITGA6 was correlated to a worse prognosis (Wu et al., 2019).

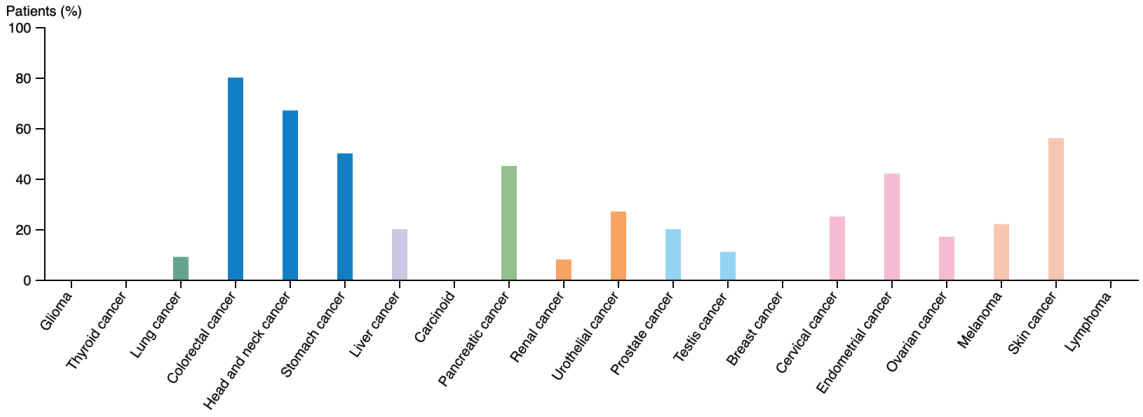

Figure 3. Immunohistochemical analysis of ITGA6 expression in various cancers using the HPA012696 antibody. Significant numbers of patients' cancers exhibit expression of ITGA6, including pancreatic cancer. Note that cancers with 0% expression do not necessarily show no ITGA6 expression, simply no reactivity with the specific antibody (The Human Protein Atlas).

Prostate Cancer

The prostate gland is a reproductive organ found only in males and is located below the bladder and in front of the rectum. The prostate functions to secrete prostate fluid, a major component of semen that nourishes and protects sperm. Muscles of the prostate gland help to propel this seminal fluid into the urethra during ejaculation. Prostate cancer begins when cells in the prostate gland develop a mutation and begin to grow out of control. Symptoms of prostate cancer include trouble urinating, blood in semen, erectile dysfunction, and discomfort in the pelvic region. About one in 9 men will be diagnosed with prostate cancer; the majority of which are adenocarcinomas, cancers that develop from the gland cells in the glandular tubes and duct lining. The death rate of men

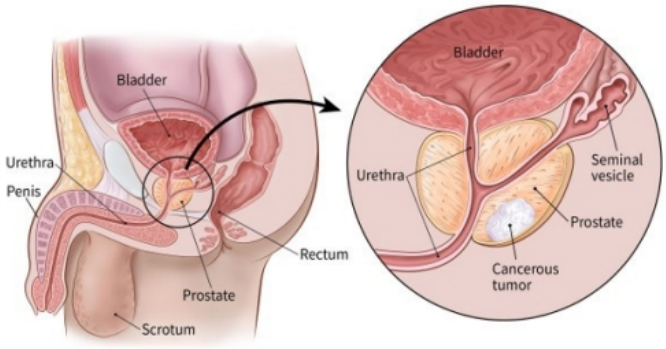

Figure 4. Anatomy of the male reproductive system. The prostate gland is responsible for producing prostate fluid and projecting this seminal fluid during ejaculation. Here, a cancer mass in the prostate is shown in white (American Cancer Society, 2020).

diagnosed with prostate cancer is 19.1%, of which 90% can be contributed to metastasis (American Cancer Society, 2020). When prostate cancer metastasizes, it is likely to invade into the bladder or even the circulatory system where it can spread to the bones or other organs. Depending on the stage of cancer progression, prostate cancer is normally treated with surgery, radiation therapy, chemotherapy, hormone therapy, or any other combination of treatments.

Present Study Aims and Significance

It was my objective to characterize the in-vitro interactions between human ITGA6 and human cancer, also to identify the mechanisms by which ITGA6 contributes to cancer metastasis. I sought to confirm my hypothesis that the decreased expression of ITGA6 would reduce cancer cell migration and that the increased expression of ITGA6 would increase cancer cell migration. By determining the functional mechanism of ITGA6 in inducing cell migration, and by experimenting with various ITGA6 constructs, identification of putative biomarkers will contribute to the discovery and design of possible drug targets. Creating chemotherapy drugs to target mutated integrin pathways have the possibility of reducing cancer deaths caused by cancer metastasis.

EXPERIMENTAL DESIGN

To understand the mechanism through which ITGA6 facilitates the migration of cancer cells through the ECM, ITGA6 was manipulated in order to test various functional pathways. Through RNA/DNA transfection, prostate cancer cells (PC3) were introduced to various human ITGA6 RNA/DNA constructs, influencing ITGA6 expression in cancer cells. Injecting human full-length ITGA6 cDNA increases expression, resulting in overexpression of ITGA6 protein. SiRNA transfection to knockdown the ITGA6 gene results in the decreased expression of ITGA6 protein. Injecting human truncated ITGA6 DNA results in the expression of cleaved ITGA6- free floating extracellular ITGA6 domains. Injecting human mutated ITGA6 DNA results in the expression of a non-cleavable ITGA6 protein. Applying these different ITGA6 constructs to PC3 cells and conducting in-vitro tests will provide insight into the mechanism by which ITGA6 contributes to cell migration.

Beginning with cultured prostate cancer (PC3) cells, the cells were split into different experimental groups transfected with different ITGA6 constructs to manipulate the cells' expression of the integrin protein. These cells were then utilized in an in-vitro laminin migration assay. Laminin is an extracellular matrix protein; the migration assay allowed us to observe the experimental groups' abilities to migrate through the ECM protein, indicating cellular aggression. Analysis of the in-vitro model provided insight regarding the functionality of ITGA6 in cancer cell migration.

METHODS

Cell Culture: Seeding a new cell line (Thawing)

First, RPMI 1640 culture medium was removed from the +4ºC refrigerator and placed in a 37 ºC water bath for 5 minutes or until warm. During this time the Biosafety Cabinet (BSC) laminar flow hood was prepared and disinfected with 70% EtOH. Additionally, all items used in the BSC hood were disinfected with 70% EtOH before entering, including pipettes, falcon tubes, cell culture plates, bottle of culture medium, etc. Next, 20mL of warmed culture medium was pipetted into two T-75 tissue culture flasks. One vial of frozen PC3 cells was removed from the Liquid Nitrogen Storage Dewar from the vapor phase and immediately placed into the 37 ºC water bath for 2 minutes. Once thawed the vial was sprayed with 70% EtOH and placed into the BSC hood. Each vial of PC3 cells contained approximately $1X10^6$ cells suspended in 1ml of cell culture medium with 10% Dimethyl Sulfoxide (DMSO); 500µL of the vial contents were pipetted into each tissue culture flask with medium. If the vial contained more than 1mL of cells, the remainder was split between the two flasks. The flasks were then gently tilted back and forth to mix the cells in the medium and then placed into the 37°C CO_2 incubator. In 48 hours, the cell culture medium in each flask was removed and the flasks were washed with 7mL 1XPBS to remove any leftover DMSO. Then 10mL of fresh culture medium was added to each flask.

Cell Culture: Harvesting cells

The cell culture medium used to sustain and grow cells was made by adding 50mL heat inactivated Fetal Bovine Serum and 5mL Penicillin/Streptomycin solution to

500mL of RPMI 1640 media. Phosphate buffered saline (10xPBS) was diluted to 1xPBS using DNase-free distilled water. Trypsin (0.05%), a serine protease, was used to dissociate adherent PC3 cells from the tissue-culture treated flasks. The culture medium and trypsin were placed a hot water bath for two minutes; during this time the BSC was sprayed with 70% EtOH. Once the bottles reached room temperature, they were disinfected along with the 1xPBS solution and placed into the BSC hood. Next, the desired flask of PC3 cells was removed from the 37°C CO_2 incubator, sprayed with 70% EtOH and placed into the BSC hood. The culture medium was then vacuumed out of the flask - careful not to touch the adherent cells on the bottom of the treated flask. 7mL of 1xPBS was added to the flask and tilted to allow the PBS to cover the entire treated surface. The 1xPBS was then vacuumed out of the flask to wash out any dead cells. 7.5mL of trypsin was then added into the flask and incubated for 5min in the 37°C CO_2 incubator. Next the flask was viewed under a compound light microscope to visualize the free-floating, detached cells. The flask was then disinfected before being placed back into the BSC hood. 2.5mL of culture medium was then added to the flask, mixing with the trypsin to inhibit its protease function. The contents of the flask were then transferred into a 50mL Falcon centrifuge tube. The Falcon tube was centrifuged at 970rpm for 5 minutes. The trypsin/medium solution was aspirated from the tube- careful not to vacuum up the mass of cells collected on the bottom of the tube. 8mL of 1XPBS was then added into the Falcon tube and was gently pipetted up and down to resuspend the cells. The Falcon tube was centrifuged again at 970rpm for 3 minutes and the PBS aspirated. 2 mL of fresh culture medium was added to the tube and cells resuspended. Finally, the number of cells were counted using a hemocytometer and the necessary concentration of cells needed for further experimentation was calculated.

Cell Labeling

Following harvesting, 1×10^6 cells were aliquoted into a microcentrifuge tube. The tube was centrifuged at 500rcf for 2 minutes. The supernatant was aspirated from the microcentrifuge tube and 1mL of serum-free medium was added. The cells were then resuspended and 5µL of DiI cell tracker (Thermo Fisher D3911) was added. A vortex mixer was used to mix the dye and then incubated for 30 minutes at 37°C. Following

incubation, the tube was re-centrifuge at 500rcf for 2 minutes. The supernatant was aspirated while being careful not to disturb the cell pellet. 200µL of RPMI 1640 medium was added and cells resuspended. The tube was incubated for another hour before microinjection into 2-day old zebrafish embryos.

ITGA6 Transfection

PC3 cells were harvested at 70%-90% confluency and seeded 0.5-2× 10^5 cells per well into a 24-well tissue culture plate. The plate was incubated for 24-48 hours before initiating transfection. In a microcentrifuge tube 4µL of Lipofectamine3000 reagent was diluted with 50µL of Opti-MEM medium. Stock solutions of ITGA6 siRNA and ITGA6 DNA were diluted to 10µM solutions. Constructs included GAPDH siRNA, siRNA s7492, and full-length DNA. In another microcentrifuge tube the constructs were diluted with 50mL of Opti-MEM and 2µL of P3000 Reagent. 2µL of GAPDH siRNA was used for GAPDH transfection, 2µL of siRNA s7492 was used for siRNA transfection, and 0.5µL of full-length DNA was used for DNA transfection. The diluted RNA/DNA constructs were then mixed with the diluted Lipofectamine 3000 Reagent in a 1:1 ratio and incubated at room temperature for 10-15 minutes. 50µL of the RNA/DNA-lipid complex was added into each well along with 0.5mL of culture medium and incubated for 48 hours at 37°C. The RNA/DNA-lipid complex mixture produced enough for two wells; the components were adjusted accordingly to account for additional experimental wells.

Laminin Migration Assay

An ECM221 QCM Laminin Migration Assay (24-well, fluorometric) kit was used to conduct an in-vitro experiment of PC3 cell migration; the following procedure was adapted from the user guide: A 10µg/mL laminin coating solution was prepared for 12 inserts by thawing one vial of laminin at room temperature for 10 minutes. The vial of laminin (60µL) was diluted with 6mL 1xPBS. A control of 0.5% BSA solution was prepared for 12 inserts by diluting 0.1mL 30% BSA with 5.9mL PBS. 0.5mL of the laminin solution, or 0.5 mL BSA solution for control wells, was added into the bottom of the receiver wells. One Boyden chamber insert was placed into each of the receiver wells

and left overnight in the +4ºC refrigerator to allow the inserts to coat in either the laminin or BSA solution.

All assay plates and reagents were warmed to room temperature and the forceps sterilized with 70% ethanol. Prior to performing the assay, using the sterilized forceps, each coated insert was washed with 1xPBS and dipped into a 10cm tissue culture plate filled with PBS. Each coated insert was then transferred into a clean receiver well filled with 0.5mL serum-free medium containing 0.5% BSA. A cell suspension containing 0.5-1×10^5 cells/mL was prepared in chemoattractant-free media containing 0.5% BSA. 200µL of the cell suspension was then added into each insert that was in contact with 0.5mL serum-free medium containing 0.5% BSA. The plate was covered and incubated for 4-24 hours at 37°C in a CO_2 incubator.

A 1mM Calcein-AM solution was prepared by dissolving 50µg Calcein-AM (one vial) with 50µL DMSO. The mixture was vortexed and briefly centrifuged. The 24-well receiver tray was removed from the incubator and 200µL Accutase cell-detachment solution was added to each well and warmed to 37°C. 1µL of the Calcein-AM solution was then added to the receiver well of the migration assay plate containing migrated cells and incubated for 15 minutes at 37°C. Next, the medium from the upper chamber of the well was removed and each insert placed into a separate well containing the Accutase cell-detachment solution. The assay plate was incubated for 30 minutes at 37°C. 100µL of each detached cell solution was transferred into the 96 well Black Quantitation Plate and the fluorescent cells viewed under wavelengths of 494/517 nm.

Cell Counting

The computer program “ImageJ” was used to quantify the number of migratory cells from the Laminin migration assay. Images of the assay results were uploaded to the program as a pdf and converted to an 8-bit file, making the image black and white: [Type]->[8-bit]. Then the contrast threshold between the white cells and the black background was adjusted to clarify the image: [Adjust]-> [Threshold]. Next, the cells that appeared to be clumped together were separated: [Binary]->[Watershed]. Finally, the number of cells were quantified: [Analyze]-> [Analyze Particles]. The minimum pixel

value was adjusted to 50 pixels in order to ignore background static that influenced the results.

Zebrafish Xenotransplantation

Cultured PC3 cells that had been radioactively labeled with DiI were loaded into the Nanoject II Auto-Nanoliter Injector. The injection needles were loaded with the labeled cells and mineral oil- which served as a pressure gradient. 2-day old zebrafish embryos were treated with 0.04% Tricaine, an anesthetic, and the embryos injected in the perivitelline space. After injection, the embryos were washed three times in E3 embryo medium to remove any remnant anesthetic. Next, the embryos were subjected to Laminin, the human ECM protein. Following incubation, the zebrafish embryos were mounted to be used for confocal microscopy imaging. The mounted embryos were placed on the laser confocal and visualized for the radioactive cancer cells within the embryo. Using the ZEN software, the cancer cells within each embryo were imaged for a set amount of time and individual cancer cell migration was tracked.

RESULTS AND DISCUSSION

Expression of ITGA6 in PC3 cells

Integrin Alpha 6 has been shown to be expressed in multiple different cancers, including pancreatic cancer. In order to confirm the expression of ITGA6 in the PC3 cell line, non-manipulated control cells were fixed and stained using a primary ITGA6 antibody from Life Technologies. Fixation with a secondary antibody marked with green fluorescent protein (Alexa Fluor 488) was used to visualize the ITGA6 in cells. Immunostaining and imaging revealed that the PC3 cell line constitutively expresses ITGA6. Their expression of ITGA6 was utilized for all experimental procedures and also employed as control cells, expressing moderate amounts of ITGA6 (Williams, 2018).

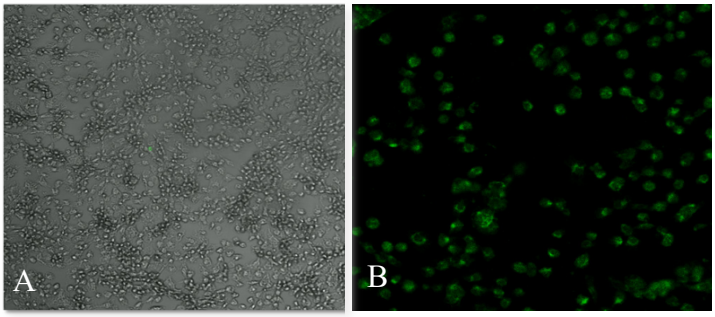

Figure 1. ITGA6 expression was visualized in the PC3 cell line via immunohistochemical analysis using a primary ITGA6 antibody and secondary antibody with GFP. A) Control PC3 cells were not stained thus do not exhibit GFP. B) PC3 cells were fixed and labeled with primary and secondary antibody and exhibit GFP (Williams, 2018).

siRNA Transfection Reduces ITGA6 Expression

In order to determine the effectiveness of siRNA on reducing the expression of ITGA6, a comparative CT analysis was performed. In order to make any conclusion regarding the hypothesis that lower levels of ITGA6 reduce cellular metastasis, the functionality of implemented siRNA needed to be confirmed. Two siRNA with different ITGA6 targets were employed in transfection: s7492 and s7493. Results from the CT analysis showed that both siRNA was able to reduce the expression of ITGA6 in PC3 cells. GAPDH siRNA was used as a positive control for comparison (Williams, 2018).

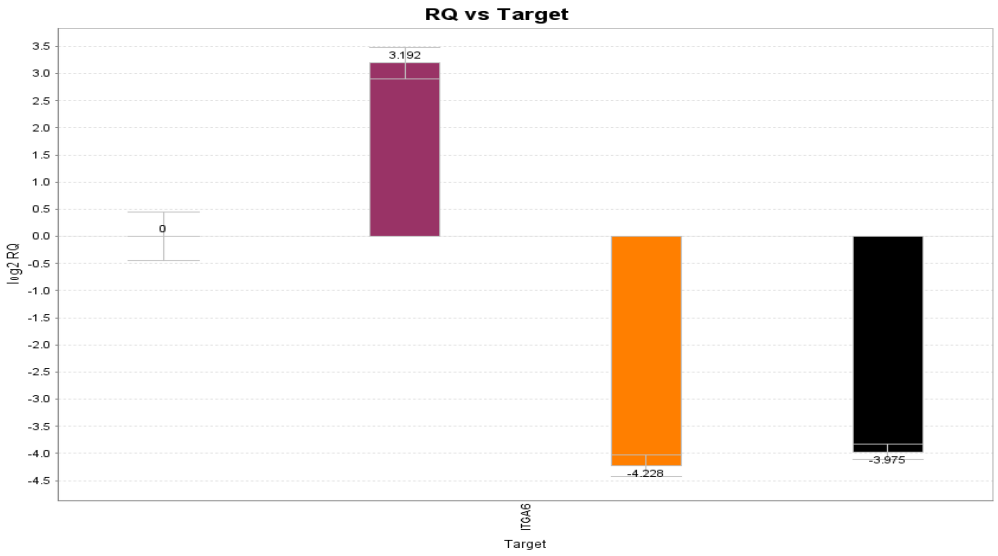

Figure 2. Comparative CT analysis indicated that both s7492 siRNA (orange) and s7493 siRNA (black) are able to reduce the expression of ITGA6 in PC3 cells. The positive control, GAPDH siRNA (magenta), did not exhibit reduced ITGA6 expression (Williams, 2018).

Laminin Migration Assay

Small interfering RNA s7492 functions to interfere with the translation of the integrin subunit alpha 6. To confirm the hypothesis that decreased expression of ITGA6 reduces the ability of cancer cells to metastasize, an in-vitro laminin migration assay was performed. PC3 cells were harvested from the stock cell line and transfected with siRNA s7492 while cells transfected with GAPDH siRNA served as a positive control to transfection; both were normalized to an untreated control group. After transfection and cell harvesting, $1.0X10^5$ cells of each group were added to both Laminin and BSA coated inserts. BSA coated wells served as a negative control to cell migration. GAPDH siRNA transfected cells and control cells were run in duplicate in both Laminin and BSA coated inserts while siRNA s7492 transfected cells were run in triplicate in both Laminin and BSA coated inserts.

To further define the role of ITGA6 in cancer cell migration, ITGA6 full-length cDNA was transfected into PC3 cells to investigate the hypothesis that an increased expression of ITGA6 increases the ability of cancer cells to metastasize. Cells transfected with FL-cDNA will express a greater amount of ITGA6 compared to control cells that express a moderate level of ITGA6. Another in-vitro laminin migration assay was performed following the transfection of PC3 cells with FL-DNA, siRNA s7492, and GAPDH siRNA. These groups were normalized to an untreated control group of PC3 cells. Due to varying levels of cell viability after transfection, the following cell counts were used for the migration assay: $9.0X10^3$ FL-DNA cells, $2.2X10^4$ siRNA s7492 cells, $5.0X10^4$ control cells, and $5.0X10^3$ GAPDH siRNA cells. For the migration assay procedure there was one set of FL-DNA experimental wells, three sets of s7492 experimental wells, one set of untreated control wells, and one set of GAPDH control wells. Laminin coated wells were compared to the negative control, BSA coated wells.

Table 1. The raw data from the migration assay (ECM221 QCM Laminin Migration Assay (24-well, fluorometric) indicating the number of migratory cells invading through the laminin coated wells and the BSA coated wells. Laminin coated wells mimic the basal lamina of the ECM in vertebrates while BSA coated wells served as a negative control to migration. Control and GAPDH cells were run in duplicate while ITGA6 knockdown cells were run in triplicate in Trial 1; ITGA6 overexpression was not tested. In trial 2 only ITGA6 knockdown cells were run in triplicate as a result of limited cell numbers. Note that the number of cells seeded for migration varied between groups and trials.

	Trial 1		Trial 2	
Cell group	BSA	Laminin	BSA	Laminin
control	14 cells / 38 cells	231 cells/ 545 cells	2 cells	527 cells
GAPDH	10/69	588/970	0	12
ITGA6 knockdown	0/19/0	80/296/37	0	197/195/158
ITGA6 overexpression	-	-	0	84

The untreated control cells experienced normal levels of migration while the s7492 transfected cells experienced 31.9% less migration. The cells transfected with siRNA s7492 had a reduced expression of the ITGA6 gene, suggesting a positive correlation between the amount of ITGA6 expressed and the ability to metastasize.

Table 2. The average percentage of migratory PC3 cells for both trials of the migration assay (ECM221 QCM Laminin Migration Assay (24-well, fluorometric). Laminin coated wells mimic the basal lamina of the ECM in vertebrates while BSA coated wells served as a negative control to migration.

Cell group	BSA	Laminin
control	$1.3x10^{-4}$ %	0.72%
GAPDH	$1.9\ x10^{-4}$	0.51
ITGA6 knockdown	$4.8\ x10^{-5}$	0.49
ITGA6 overexpression	0	0.93

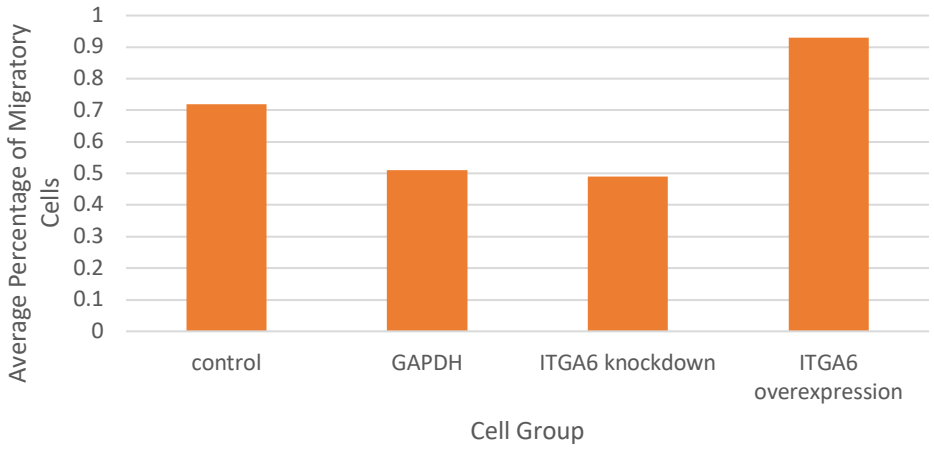

Figure 3. A bar graph of the average percentage of migratory PC3 cells for both trials of the migration assay (ECM221 QCM Laminin Migration Assay (24-well, fluorometric) in the Laminin coated wells. ITGA6 knockdown cells exhibited the least amount of expression, followed by the control PC3 cells, and then the ITGA6 overexpression cells which exhibited the greatest amount of migration. Note that the migration results of the BSA coated wells were excluded due to the insignificant migration rates.

The percentage of migratory cells was calculated by dividing the number of cells that had migrated through the Laminin or BSA coated inserts by the total number of seeded cells and multiplied by one hundred (**Table 2**). The siRNA transfected cells provided consistent results that the knockdown of ITGA6 reduces PC3 cell migration. ITGA6 knockdown decreased cell migration by 0.23% compared to control PC3 cells. My hypothesis that an increased expression of ITGA6 would increase cellular metastasis was supported by the evidence that the FL-DNA transfected cells experienced a 0.21% increase in migration compared to control PC3 cells; However, there are multiple constraints within the experimental setup. During the transfection procedure, a considerable amount of cell death was noticed for the ITGA6 FL-cDNA cells, presumably due to the addition of too much DNA. Due to the limited number of cells, the number of cells used for the migration assay was not consistent between groups. Additionally, it is possible that the FL-cDNA transfected into the PC3 cells did not take, providing inaccurate results concerning the percentage of migratory cells.

FUTURE RESEARCH

Integrin Alpha 6 expression has been associated with metastasis in multiple different cancers, specifically prostate cancer. The decreased expression of ITGA6 in PC3 cells via siRNA transfection reduced the ability of cancer cells to migrate in-vitro. Furthermore, the increased expression of ITGA6 in PC3 cells via FL-cDNA transfection increased the ability of cancer cells to metastasize, to a slight extent. Further trials with ITGA6 FL-cDNA transfected PC3 cells need to be repeated to collect additional data regarding increased migration. Similar in-vitro experiments should be performed with various truncated and cleaved forms of ITGA6 to identify the functional unit controlling ECM remodeling, thus facilitating the delamination and migration of cancer cells. In-vivo experiments using zebrafish would strengthen any additional results collected from in-vitro experimentation and serve as a basic proxy compared to a human system. A better understanding of the functional mechanism by which ITGA6 initiates and facilitates cancer metastasis is of high priority to identify potential drug targets to prevent cancer metastasis.

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